free IF3 is a highly flexible molecule. However this flexibility is lost completely after binding to the 30s subunit of the ribosome where it functions as a structural stabiliser, supporting the conformer selection model for ligand binding.
Human guanylate binding protein 1 (hGBP1) is a member of the dynamin superfamily which is known for mediating membrane fusion in endocytosis. We show fast dynamic processes in solution between two distinct conformations. After binding its substrate GTP, hGBP1 becomes more rigid, working as a conformational switch where the GTP-bound conformation allows dimerisation leading to a functional dimer.
DNA polymerases, such as the Klenow fragment of DNA polymerase I, are highly processive enzymes synthesizing double stranded DNA with a rate constant, kpol, of ~40 s-1. We could show fast dynamics between different conformations of the enzyme in the absence of substrate. After binding of DNA and the ''correct'' dNTP, a closure of the protein is expected but however was not seen, rather the equilibria is shifted towards a closed conformation. This flexibility reflects the balance between specificity and velocity for this polymerase.

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Fluorescence Correlation Lifetime Spectroscopy Study of Xanthene Derivatives in Water-In-Oil Micelles
Angel Orte, Maria Jose Ruedas-Rama, Jose M. Paredes, Luis Crovetto, Eva M. Talavera, Jose M. Alvarez-Pez.
Fluorescence Lifetime Correlation Spectroscopy (FLCS) allows to extract autocorrelation functions from different emitters by using Time-Correlated Single Photon Counting information, extending the applicability of conventional Fluorescence Correlation Spectroscopy (FCS) [1].
Here, we have used a xanthene derivative, so-called Tokyo Green II (TG-II) [2,3], to study the size, diffusional properties, and dynamics of reverse water-in-oil micelles of Aerosol-OT (AOT) in isooctane (iC_8). The dye undergoes a partition between the inner micellar aqueous core, in which the TG-II anion is the main form, and the AOT lipophilic side chains, where the TG-II neutral species is predominant. By applying FLCS we obtained the lifetime filtered autocorrelation (AC) curves of the anionic TG-II, which shows a characteristic lifetime of 3.70 ns, under different conditions. Fitting the TG-II anion AC curves to the appropriate model provided the diffusion coefficient and hydrodynamic radius of micelles at different ratios [water] / [AOT]. Interestingly, the AC functions also showed a strong anticorrelation time in the microsecond time range. This time represents micelle dynamics that changes the distribution of the TG-II neutral and anionic forms in the hydrophobic interface and the water core.
[1] Kapusta et al., *J. Fluoresc.***17** (2007), 43.
[2] Paredes et al., *Phys. Chem. Chem. Phys.***11** (2009), 5400.
[3] Paredes et al., *Phys. Chem. Chem. Phys.***12** (2010), 323.

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Imaging Total Internal Reflection Fluorescence Correlation Spectroscopy (ITIR-FCS) Detects Multiple Lipid Domains on Live Cell Membranes
Thorsten Wohland, Jagadish Sankaran, Manoj Manna, Rachel Susan Kraut.
The cell membrane consists of a large number of different lipid molecules, of membrane spanning and associated proteins and is mechanically linked to the cytoskeleton and the extracellular matrix. It exhibits a complicated, highly dynamic structure which reflects its large number of constituent components and which is essential for signalling and trafficking in live cells. This structure is thought to have different characteristic length scales which can range from tens to hundreds of nanometers. Thus new methods are needed which can provide information on membrane structures at these different length scales with high temporal resolution. Imaging Total Internal Reflection Fluorescence Correlation Spectroscopy (ITIR-FCS) provides fluorescence correlation measurements on each point of a whole cell membranes with diffraction limited resolution and a better than millisecond time resolution. In particular the differences between spatial cross-correlation measurements, so-called ΔCCF, allows to infer changes in membrane organization and in combination with the diffusion coefficients provides information about membrane dynamics, organization and their changes during drug treatment. Here, in a combination of simulations, measurements on pure and mixed lipid supported bilayers, and live cell membranes stained with domain markers, we show that cell membrane composition is a crucial determinant of the type of domains existing at any point in time. By using markers for liquid ordered (sphingolipid binding domain, SBD) and disordered (DiIC18) lipid phases and a range of membrane composition- and cytoskeleton-influencing agents (methyl-β-cyclodextrin, latrunculin A, NB-DNJ, fumonisin B1, sphingomyelinase) we demonstrate that we have at least two different, ceramide and sphingolipid dependent, domain types, and that membrane fluidity and organization are not necessarily correlated and exist over different length scales.

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3D Molecular Tracking in Live Cells with Simultaneous Time-Resolved Spectroscopy
James Werner, Peter Goodwin, Elizabeth Phipps, Patrick Cutler, Diane Lidke, Bridget Wilson.
We report a confocal feedback method for tracking the motion of individual quantum dot labeled proteins as they move in three dimensions inside of cells that has sub-nanosecond temporal resolution. 3D tracking molecular is possible for tens of microns in X, Y, and Z, meaning tracking can occur throughout the entire cell volume volume for many cell lines. The sub-nanosecond temporal resolution enables time-resolved spectroscopies (e.g., fluorescence lifetime measurements or fluorescence correlation spectroscopy) to be made on the molecules as they are being tracked. In particular, recording of the arrival times of individual photons enabled, for the first time, photon pair correlation measurements showing fluorescence photon anti-bunching of individual QD labeled proteins in live cells. The power of this new technology is further illustrated through tracking of individual QD-labeled IgE receptor complexes on rat mast cells, revealing three-dimensional nano-scale topology of the cell membrane as individual receptors navigate hills and valleys of a dynamically changing plasma membrane landscape. In addition to mapping out cell surface topology, IgE-Fc{epsilon}RI signaling clusters were also captured in the act of ligand-mediated endocytosis and tracked during rapid (~950 nm/s) vesicular transit through the cell.

2574-Pos Board B560
Nano-Positioning-System Provides Structural Insights into ATP-Dependent Nucleosome Remodeling
Barbara Treutlein, Adam Muschielok, Daniel Ryan, Tom Owen-Hughes, Jens Michaelis.
The packaging of chromosomal DNA by nucleosomes condenses and organizes the genome, but occludes many regulatory DNA elements. In order to allow central nuclear processes such as DNA replication, recombination, repair and transcription, cells have evolved so-called chromatin remodeling complexes, which use the energy of ATP hydrolysis to move, destabilize, eject, or restructure nucleosomes.
Chd1 (Chromodomain-helicase-DNA-binding protein 1) is a single-subunit chromatin remodeler consisting of three domains, namely the characteristic tandem-chromodomain, the helicase domain and the DNA-binding domain. It has been shown to assemble, slide and space nucleosomes in vitro and associate with actively transcribed regions of the genome in vivo, but the molecular mechanism of Chd1 remodeling is still not well understood.
Single-molecule (sm) techniques such as sm-FRET allow the direct and real-time investigation of conformational changes within macromolecular complexes and hence are perfectly suited to study the mechanism of molecular processes such as chromatin remodeling.
Here, we apply sm-FRET and the recently developed Nano-Positioning-System (NPS) [1] to determine the conformation of Chd1 bound to a mononucleosome. Briefly, we determine the distances between several known positions on the nucleosome to unknown positions on different domains of Chd1. The NPS computes the 3-dimensional position probability densities of the sites on Chd1 relative to the nucleosome. By combining our position probability densities with data from SAXS and x-ray crystallography on the structure of the different Chd1 domains, a structure of Chd1 bound to a mononucleosome can be modeled.
[1] Muschielok et al., Nature Methods, 5, 965 (2008).

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Nano-Positioning-System Reveals Structure of the RNA Polymerase II Initially Transcribing Complex (ITC)
Barbara Treutlein, Adam Muschielok, Joanna Andrecka, Anass Jawhari, Claudia Buchen, Friederike Hög, Patrick Cramer, Jens Michaelis.
Transcription initiation is a multi-step process requiring binding of RNA polymerase II (Pol II) to the promoter, melting of DNA and its insertion into the active site. Pol II needs the assistance of several transcription factors (TFs), most essentially the TATA-binding protein (TBP), TFIIB and TFIIF.
X-ray crystallography has provided detailed insights into the architecture of Pol II in different functional states, but structural studies of transcription initiation have been hindered by the flexibility of Pol II initiation complexes. Recently, the structure of a Pol II-TFIIB complex was solved [1] indicating the mechanism of transcription initiation and providing models for closed and open complex. However, the structure of a Pol II initiation complex including all involved TFs is still missing.
Here, we employ the recently developed Nano-Positioning-System (NPS) [2] to unravel the structure of a Pol II ITC, consisting of Pol II, TFIIF, TBP, TFIIB